



EP041785



INVESTOR IN PEOPLE

The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QD

REC'D 02 JUN 2004

WIPO

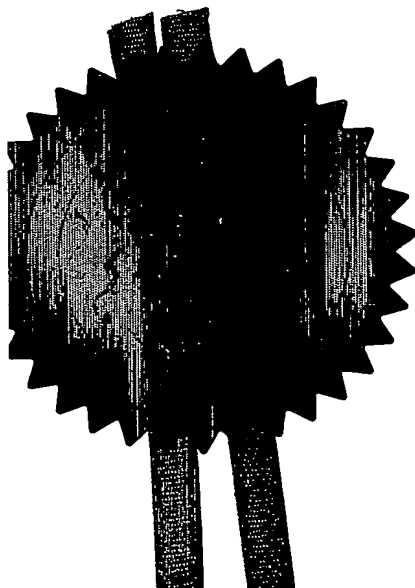
PCT

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.



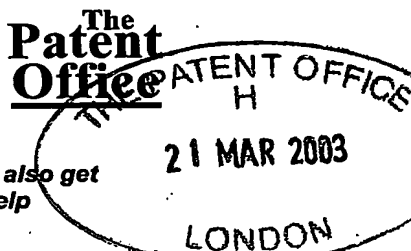
Signed

Dated 1 March 2004

**PRIORITY
DOCUMENT**

SUBMITTED OR TRANSMITTED IN
COMPLIANCE WITH RULE 17.1(a) OR (b)

Patents Form 1/77

Patents Act 1977
(Rule 16)

1/77

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office

Cardiff Road
Newport
Gwent NP9 1RH

1. Your reference	RMW/T3099(C)	21 MAR 2003
2. Patent application number (The Patent Office will fill in this part)	0306568.7	24MAR03 E794295-7 C03008 P01/7700 0.00-0306568.7
3. Full name, address and postcode of the or of each applicant (underline all surnames)	Unilever PLC Unilever House Blackfriars London EC4P 4BQ GB	1628002
Patents ADP number (if you know it)		
If the applicant is a corporate body, give the country/state of its incorporation	GB	
4. Title of the invention	COMPOSITIONS OF NATURAL PRODUCTS AND USE THEREOF	
5. Name of your agent (if you have one) "Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)	LLOYD WISE COMMONWEALTH HOUSE 1-19 NEW OXFORD STREET LONDON WC1A 1LW ENGLAND	
Patents ADP number (if you know it)	117001	✓
6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications (and if you know it) the or each application number	Country	Priority application number (if you know it)
		Date of filing (day / month / year)
7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application	Number of earlier application	Date of filing (day / month / year)
8. Is a statement of inventorship and of right to grant of a patent required in support of this request (Answer 'Yes' if: a) any applicant named in part 3 is not an inventor, or b) there is an inventor who is not named as an applicant, or c) any named applicant is a corporate body. See note (d))	YES	

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form.

Do not count copies of the same document

Continuation sheets of this form

Description 7 ✓

Claim(s) 2 ✓

Abstract 1 ✓

Drawing(s)

10. If you are also filing any of the following, state how many against each item.

Priority documents

Translation of priority documents

Statement of inventorship and right to grant of a patent (*Patents Form 7/77*)

Request for preliminary examination and search (*Patents Form 9/77*) 1 ✓

Request for substantive examination (*Patents Form 10/77*)

Any other documents
(please specify)

11. I/We request the grant of a patent on the basis of this application.

Signature

Date 21 March 2003

12. Name and daytime telephone number of person to contact in the United Kingdom

ROBIN MICHAEL WALDREN
020 7571 6200

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

a) If you need help to fill in this form or you have any questions, please contact the Patent Office on 0645 500505.

b) Write your answers in capital letters using black ink or you may type them.

c) If there is not enough space for all relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.

d) If you have answered 'Yes' Patents Form 7/77 will need to be filed.

e) Once you have filled in the form you must remember to sign and date it.

f) For details of the fee and ways to pay, please contact the Patent Office.

COMPOSITIONS OF NATURAL PRODUCTS AND USE THEREOF

Field of the Invention

5 The present invention relates to compositions of hops extracts and isoflavones.

Background of the Invention

Isoflavones are known as compounds that can be applied to prevent or treat many
 10 health deficiencies or to achieve certain health effects not directly related with a health
 deficiency. For example, they are known to achieve benefits in the women's health
 area in particular for postmenopausal women. These effects are disclosed in e.g. US 5
 498 631; WO 98/ 56373; WO 98/08503; US 5 733 926; US 5 952 374 and many other
 references. Health effects that are also attributed to isoflavones include skin effects
 15 and anti-inflammatory effects.

EP-A-1 180 331 discloses blends of quercetin (a flavone) and certain isoflavones as
 anti-inflammatory agents and anti-ageing agents for the skin.

20 On the other hand, certain compounds found in hops, namely xanthohumol and iso-
 xanthohumol have been reported as having anti-inflammatory and skin benefit
 properties when incorporated in food products, as disclosed in EP-A-1 190 630. Our
 International Patent Application No. PCT/EP02/03412, unpublished at the priority date
 of the present invention, also describes the same properties for 8-prenylnaringenin (8
 25 pn). However, as these compounds are potentially oestrogenic, it would be desirable if
 other non-oestrogenic compounds could be found in hops to fulfil the same function.

We have now found that the iso-alpha acids found in hops and hop extracts can act
 synergistically in combination with certain isoflavones for obtaining anti-ageing and
 30 anti-inflammatory effects.

Definition of the Invention

A first aspect of the present invention now provides a composition comprising at least one iso-alpha acid hops extract and at least one of natural isoflavones, wherein the at least one of the isoflavones is selected from genestein, daidzein and glycerin either in the glucon or in the aglucon form and wherein quercetin and the natural isoflavone are present in weight ratios of 1:50 to 50:1, preferably 20:1 to 1:20, more preferably 1:6 to 6:1, most preferably 1:4 to 4:1, calculated as aglucon, while genestein and daidzein are present in a weight ratio (as aglucon) of 2:1 to 1:2.

Detailed Description of the Invention

In compositions according to the present invention the weight ratios hops alpha acids to isoflavone can vary over a wide range. However, it is generally preferred to have a weight ratio in the range from 1:50 to 50:1, preferably ratios of 1:20 to 20:1, more preferably ratios of 1:6 to 6:1 and even more preferably from 1:4 to 4:1 and most preferably from 1:2 to 2:1, calculated as aglucon, were applied. Even better results were obtained by using weight ratios of 1:2 to 1:1.

20

The most preferred isoflavones in these compositions are genestein and daidzein (or as glucons the genistin and daidzin). Although these components can be applied in a wide range of ratios the best results were obtained, when the genistein and daidzein were used in weight ratios of 2:1 to 1:2.

25

The hops alpha acids that can be applied according to the invention are suitably derived from natural sources. Hops contain, *inter alia*, alpha, beta and gamma acids. Iso-alpha acids are a particular isomeric form of the α acids. The hops iso-alpha acids are commercially available as purified isohop extract from Botanix Ltd. The hop acid part of the soft resin fraction contains the related α acids and β acids. The γ resins are hard, and contribute nothing to the brew. The most important are the α acids, or humulones, which are characterised as humulone, cohumulone, ad humulone, pre humulone and post humulone. The relative amount of ad humulone is fairly constant

30

between varieties whilst the relative amounts of humulone and cohumulone are variety dependent, and this can from a technical standpoint, create substantial quality differences.

- 5 The iso-alpha acid hop extracts are commercially available in forms which may actually contain a mixture of molecular species. In practice, the efficacy of the various iso-alpha acids in combination with the isoflavones varies. Thus, especially preferred are those extracts which comprise or consist of the lowest 75%, preferably the lowest 40% by weight of fractionated total hops iso-alpha acids extract eluted with a solvent mixture
10 consisting of n-heptane/isopropanol/formic acid in a weight ratio of 90:15:0.5.

- Although use of the isolated iso-alpha acid hop extracts avoid the potentially oestrogenic content of the hops, also within the scope of the present invention are compositions which comprise total hops concentrates and/or hops extracts containing
15 substantial amounts of other materials found in hops in combination with the relevant isoflavones and/or natural sources of those isoflavones (such as soy and/or clover sources) and/or concentrates of same.

- The application of above teaching might result in a blend wherein the hops alpha acid
20 content is present in amounts of 10mg to 200 mg per RDI (recommended daily intake) while the isoflavones can be present in amounts of 10mg to 200mg per RDI. In this way the health components can be delivered as part of the daily servings of the food product.

- 25 According to another embodiment of our invention the invention also concerns food products containing a health component (= Functional food) wherein the food product comprises an amount of the composition according to the present invention, so that the total recommended daily intake (= RDI) of the health components is delivered by one to 5 servings per day of the food product.

30

Typically the food products can be selected from the group consisting of nutritional supplements, spreads, margarines, creams, sauces, dressings, mayonnaises, ice creams, fillings, confectioneries, health bars, cereals, health drinks.

In these food products 20 to 400 mg recommended daily intake of the synergistic compositions according to the invention can be present. Because of the occurrence of the synergy the food product can contain less of the individual hops iso-alpha acid(s) extracts and iso-flavones, than otherwise would be required to achieve similar effects. In this way the performance of the food product is not negatively affected by the presence of the synergistic blend of health components while the health benefits are obtained.

- 10 In addition to the above components the blends and the food products can contain other micronutrients, examples thereof being anti oxidants (Vitamin C or Vitamin E), other vitamins in particular Vitamin B1, B6 and B12, Vitamin K, folic acid, minerals like calcium, magnesium, iron, copper, or zinc, however, emulsifiers also can be present as well as minor amounts of polyunsaturated fatty acids in particular DHA and EPA and in particular (deodorised) fish oils or concentrates thereof.

Compositions according to the present invention and food products containing them can be used to achieve certain health effects, in particular certain cosmetic effects. Therefore our invention also concerns the use of a composition comprising one or more hops iso-alpha acids and one or more isoflavones as hereinbefore defined, wherein the composition or food product is applied to achieve cosmetical effects, in particular skin benefits and skin related effects such as anti-ageing effects or for promoting the formation of collagen or for promoting the decorin formation in the skin. Further the invention concerns the use of a health composition comprising hops iso-alpha acid extracts and isoflavones wherein the health composition is the blend according to the invention and wherein the blend is applied for the production of a functional food with anti-inflammatory properties with a synergistic effect on the anti-inflammatory and related health properties.

- 30 This use can also result in a method for the treatment respectively the prevention of inflammations and related health deficiencies in animals or humans by administering to the animal or human in one or more servings in total an effective amount of a

composition according to the invention or of the food product containing such a composition.

However the method can also comprise a method to achieve skin benefits or skin related effects, respectively to achieve the promotion of collagen formation or decorin formation in the skin by administering to an animal or human in one or more servings per day in total an effective amount of a composition according to the invention or a food product containing such a composition.

- 10 In these methods for administering the amount to be administered should be the effective amount of the health component corresponding with the recommended daily intake of the isoflavones and hops iso-alpha acid(s).

Examples

15

Fibroblasts PGE₂ Assay

- PGE₂ production by human skin fibroblasts can be induced by the inflammatory stimulus PMA (phorbol myristate acetate). PMA represents an external stressor which induces oxidative stress and inflammatory responses in cells. This model is used to model inflammation *in vivo*.

- Primary human foreskin fibroblasts at passage 2 (P2) were seeded into 96-well plates at 35,000 cells/well and maintained for 24 hours in an atmosphere of 5% carbon dioxide in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% foetal calf serum. Novasoy 40^R containing 20 mM isoflavones, genistein, daidzein and glycerin (in a ratio of 1:1.3:0.3) and 5mM quercetin were, either independently or in combination added to the cells (DMEM, supplemented with 10% foetal calf serum) in dimethylsulphoxide (ethanol, final concentration 1%) in triplicate and incubated for a further 24 hours. Phorbol myristate acetate (PMA) (Sigma) was added to the media and the cells incubated for a further 24 hours. The control did not contain any test compounds nor any PMA. The fibroblasts/media were then analysed immediately or snap frozen in liquid nitrogen and stored at -70°C for future analysis. The cells were

then counted and data from the dot-blot analysis subsequently standardised to cell number.

Prostaglandin E₂ (PGE₂) assay: Volumes of 50µl culture medium were taken for PGE₂ assay after gently shaking the culture plate. PGE₂ levels in the medium were determined with a Biotrak PGE₂ immunoassay kit (Amersham, UK). The assay is based on the competition between unlabelled PGE₂ in the sample and a fixed quantity of horseradish peroxidase labeled PGE₂ for a limited amount of fixed PGE₂ specific antibody. Concentrations of unlabeled sample PGE₂ are determined according to a standard curve, which was obtained at the same time.

When hops alpha acids and Novasoy 40 are added to cells at sub-optimal concentrations (i.e. those concentrations just below the concentrations that produce maximal down-regulation of PGE₂) the down-regulation of PGE₂ can be enhanced when hops iso-alpha acids and 5.4µg/ml of isoflavones from Novasoy 40 are combined at a concentration of 300µM (isoflavones), respectively. A synergistic interaction was observed between Novasoy 40 and quercetin when they were combined at the above concentrations, thus:-

20

		PGE ₂ ng/ml	PGE ₂ % total
25	Control	1246.1	
	Control +veh	1191.6	
	Control +veh +PMA	12800.0	100.0
	Iso-alpha acids 300µg/ml	3851.0	30.1
	Novasoy 5.4µg/ml	12800.0	100.0
30	Iso-alpha acids 1/1000/Novasoy 20µM	1164.5	9.1

35

Example

Soup Composition

- 5
- 3.4 grams of vegetable fat }
 - 0.5 g of modified egg yolk } together named "creamer"
 - 6.0 g of maltodextrin }
 - 0.025 grams of Novosoy 40^R containing 40 wt % of the isoflavones genistin / daidzin
- 10 and glycitin in a weight ratio of 1.3 : 1 : 0.3
- 0.000446 grams of hops iso-alpha acids
 - 0.6 grams of maize starch croutons
 - 16.1 .grams of dried potato starch
 - 1.0.grams of salt
- 15 0.3 grams of onion solids
- 0.7 grams of onions
 - 0.2 grams of parsley and herb extract
 - 3.2 grams of flavouring agents
- 20 The creamer and the other components are mixed in mixer The blend obtained is a dried instant onion soup that can be used for making a soup by mixing it with 200 ml of boiling water under stirring.

Claims

1. A composition comprising at least one iso-alpha acid hops extract and at least one of natural isoflavones, wherein the at least one of the isoflavones is selected from genestein, daidzein and glycitin either in the glucon or in the aglucon form and wherein quercetin and the natural isoflavone are present in weight ratios of 1:50 to 50:1, preferably 20:1 to 1:20, more preferably 1:6 to 6:1, most preferably 1:4 to 4:1, calculated as aglucon, while genestein and daidzein are present in a weight ratio (as aglucon) of 2:1 to 1:2.
2. A composition according to claim 1, wherein the weight ratio of total iso-alpha acid hops extract to total isoflavones is in the range from 1:2 to 2:1, preferably from 1:2 to 1:1.
3. A composition according to any preceding claim, wherein the at least one iso-alpha acid hops extract comprises or consists of the fraction with the lowest 75%, preferably the lowest 40% by weight mobility of fractionated total hops iso-alpha acids extract eluted with a solvent mixture consisting of n-heptane/isopropanol/formic acid in a weight ratio of 90:15:0.5.
4. A composition according to any preceding claim, wherein the natural isoflavones are derived from soy and in particular comprise soy flour or soy extracts, preferably soy extracts with an increased content of isoflavones.
5. A composition according to any preceding claim, wherein the blend contains 10mg to 200mg per RDI of the iso-alpha acid hops extract and 10mg to 200mg per RDI of the isoflavones named in claims 1 to 4.
6. A food product wherein the food product comprises an amount of the blend of at least one iso-alpha acid hops extract and at least one of the isoflavones genestein, daidzein and glycitin according to any preceding claim, so that the total recommended daily intake (= RDI amount) of the health components is delivered by one to 5 servings per day of the food product.

7. A food product according to claim 6, wherein the food product is selected from the group consisting of spreads, margarines, creams, sauces, dressings, mayonnaises, ice creams, fillings, confectioneries, health bars, cereals and health drinks.
8. A food product according to claim 6 or claim 7 wherein the food product contains 20mg to 400mg per recommended daily intake of the synergistic blend according to claims 1 to 7.
9. Use of a health composition comprising a composition according to any of claims 1 to 5 or a food composition according to any of claims 6 to 8 and wherein the blend or food product is applied to achieve skin benefits and skin related effects such as anti-ageing effects or for promoting the formation of collagen or for promoting the decorin formation in the skin.
10. Use of a health composition comprising a composition according to claims 1 to 5, wherein the blend is applied for the production of a functional food with anti-inflammatory properties.
11. A method for the treatment respectively the prevention of inflammations and related health deficiencies in animals or humans by administering to the animal or human in one or more servings in total an effective amount of a composition according to any of claims 1 to 5 or of a food product according to claims 6 to 8.
12. A method to achieve skin benefits or skin related effects, respectively to achieve the promotion of collagen formation or decorin formation in the skin by administering to an animal or human in one or more servings per day in total an effective amount of a composition according to claims 1 to 5 or a food product according to any of claims 6 to 8.
13. A method according to claim 11 or claim 12, wherein the effective amount of the health component corresponds with the recommended daily intake of the iso-alpha acid hops extracts and of the isoflavones.

ABSTRACT**COMPOSITIONS OF NATURAL PRODUCTS AND USE THEREOF**

Blends of hops iso-alpha acids and isoflavones from the group consisting of genestein, daidzein and glycerin display synergistic effects when applied as anti-inflammatory agent or as skin agent in particular for anti ageing purposes.